Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy cows

Denholm, SJ; McNeilly, TN; Banos, G; Coffey, MP; Russell, GC; Bagnall, A; Mitchell, MC; Wall, E

Published in:
Journal of Dairy Science

DOI:
10.3168/jds.2016-11679

Print publication: 01/04/2017

Document Version
Peer reviewed version

Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy cows.

By Denholm et al. Dairy cow health and fertility represent a major constraint on production and are significant causes of poor welfare. Recently, there has been a growing interest in identifying immune-associated and immune-response traits in livestock which are linked with disease conditions. Our results provide evidence that cellular immune-associated traits are heritable and the noticeable variation between animals would permit selection for altered trait values. Results also show that genetic selection for cellular immune-associated traits could lead to a useful tool in improving animal health, fitness and fertility. Our work expands on previous results and adds to the growing area of identifying measurable immune related traits to act as markers for health and welfare in livestock systems.

GENETIC PARAMETERS OF IMMUNE-ASSOCIATED TRAITS

Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy cows

Scott J. Denholm,*[1] Tom N. McNeilly,† Georgios Banos,*‡ Mike P. Coffey,* George C. Russell,† Ainsley Bagnall,* Mairi C. Mitchell,† and Eileen Wall*

*Scotland's Rural College (SRUC), Kings Buildings, West Mains Road, Edinburgh, EH9 3JG, United Kingdom

†Moredun Research Institute, Pentlands Science Park, Midlothian EH26 0PZ

‡The Roslin Institute, The University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, United Kingdom

Scott John Denholm

+44 (0) 131 651 9344

scott.denholm@sruc.ac.uk

ABSTRACT

Data collected from an experimental Holstein-Friesian research herd were used to determine genetic and phenotypic parameters of innate and adaptive cellular immune-associated traits. Relationships between immune-associated traits and production, health and fertility traits were also investigated. Repeated blood leukocytes records were analyzed in 546 cows for 9 cellular immune-associated traits, including %T cell subsets, B cells, NK cells and granulocytes. Variance components were estimated by univariate analysis. Heritability estimates were obtained for all 9 traits, the highest of which were observed in the T cell subsets % $CD4^+$, % $CD8^+$, $CD4^+$:$CD8^+$ ratio and % $NKp46^+$ cells (0.46, 0.41, 0.43 and 0.42, respectively) with between-individual variation accounting for 59% to 81% of total phenotypic variance. Associations between immune-associated traits and production, health and fertility traits were investigated with bivariate analyses. Strong genetic correlations were observed between % $NKp46^+$ and stillbirth rate (0.61), and lameness episodes and % $CD8^+$ (-0.51). Regarding production traits, the strongest relationships were between $CD4^+$:$CD8^+$ ratio and weight phenotypes (-0.52 for liveweight; -0.51 for empty bodyweight). Associations between feed conversion traits and immune-associated traits were also observed. Our results provide evidence that cellular immune-associated traits are heritable, and repeatable, and the noticeable variation between animals would permit selection for altered trait values, particularly in the case of the T cell subsets. The associations we observed between immune-associated, health, fertility and production traits suggest that genetic selection for cellular immune-associated traits could provide a useful tool in improving animal health, fitness and fertility.

Keywords: dairy cow, immune-associated trait, heritability, variance

INTRODUCTION

Dairy cow health represents a major constraint on production and is a significant cause of poor welfare. This is particularly true in the case of the modern high-yielding dairy cow where periods such as early lactation carry a heightened risk of disease and susceptibility to mastitis and other mammary infections is increased (Collard et al., 2000; McDougall et al., 2007). Genetic selection for increased milk yield has been highly successful, however, it has also resulted in unforeseen negative impacts on health, longevity and production (Pryce et al., 2004; Oltenacu and Broom, 2010; Koeck et al., 2013; Pritchard et al., 2013). The ability to predict the occurrence of disease in dairy cows is crucial in maintaining a high level of production within a herd as well as ensuring any financial loss is kept to a minimum (Huijps et al., 2008). Two examples of approaches to improve dairy cow health are to identify phenotypic markers (*i.e.,* biomarkers) which can be used to predict the occurrence of health events and allow early intervention, and/or to identify heritable traits which are associated with improved health function for use in future genetic selection programs aimed at reducing disease incidences and health conditions. Recently, there has been a growing interest in identifying and measuring immune-associated (**IA**) phenotypes in livestock which could then be associated with disease/health conditions. Such IA phenotypes could be used to estimate an individual's susceptibility to disease and/or act as biomarkers of concurrent disease (Park et al., 2004; Clapperton et al., 2005, 2008, 2009; Flori et al., 2011a; b; Thompson-Crispi et al., 2012a; b; van Knegsel et al., 2012; Banos et al., 2013). Previous research has looked at either steady state measurements such as circulating leukocyte populations, acute phase proteins and serum cytokine levels (Park et al., 2004; Glass et al., 2005; Clapperton et al., 2005, 2008; Flori et al., 2011a; b; Banos et al., 2013), or *in vitro* measurements of immune responsiveness focusing on innate and adaptive arms of the immune response (Thompson-Crispi et al., 2012a; b; Heriazon et al., 2013; Thompson-Crispi et al., 2014b; Mallard et al., 2015). Moreover, it has been suggested that including measurable immune response (**IR**) phenotypes in selection indices may be a viable option in

decreasing disease and improving animal health (Abdel-Azim et al., 2005; Thompson-Crispi et al., 2012a; Mallard et al., 2015)

Previously, using a cohort of 248 lactating Holstein-Friesian dairy cows sampled repeatedly over a ten month period, we identified a number of cellular IA traits within the circulating leukocyte population which were significantly associated with important health, fertility and lactation traits, including mastitis, lameness and infertility. These included a negative association between the $CD4^+$:$CD8^+$ T lymphocyte ratio and subclinical mastitis, a negative association between % $CD8^+$ T lymphocytes within the total circulating leukocyte population and fertility, and a positive association between % $NKp46^+$ leukocytes and lameness. Furthermore, these cellular measures were highly repeatable and displayed significant between-animal variation, suggesting they may be altered by genetic selection (Banos et al., 2013).

The aim of the present study was to add to the previous findings by using a larger dataset, and corresponding pedigree information, to estimate genetic and phenotypic variance components for various subsets of blood leukocytes. Further, we investigate the genetic and phenotypic associations between these cellular IA traits and health, fertility, production and functional traits (e.g., somatic cell count, feed intake, liveweight, body condition score) in dairy cows.

MATERIALS AND METHODS

Animals

All animals in the study population were Holstein-Friesians from the Langhill lines of dairy cattle housed at the SRUC Dairy Cattle Research Centre at Crichton Royal Farm, Dumfries in Scotland. Cows were born between January 2003 and September 2012 and were between their 1st and 5th lactation (inclusive). Cows in the Langhill herd are routinely and extensively monitored for productivity, health, welfare and reproduction, generating a wealth of phenotypic data for use in statistical analyses. Full pedigree spanning seven generations was available.

Langhill cows are involved in an on-going selection experiment in a 2 by 2 approach (genetic line x feeding systems) that has been running for over 30 years (Veerkamp et al., 1994). Cows are divided equally between two genetic groups, a control and a select. Those in the control group were daughters of sires selected with the UK average genetic merit for milk fat and protein. In contrast, cows in the select group were from sires selected with the highest genetic merit for milk fat and protein (Pryce et al., 1999; Bell et al., 2011). Within each genetic group cows were also divided among two distinct feed groups that aimed to be divergent in terms of energy content. From 2002 to 2009 animals were split between an indoor non-grazing/low forage system with a target ME of 12.3 MJ/kg DM with the other half of the herd receiving a high forage diet with summer grazing with a target ME of 11.5 MJ/kg DM. From September 2009 cows moved to different diets, either a home-grown forage diet (home-grown) or on a bought-in by-product feed (by-product). Over summer, the animals on the home-grown forage diet were at grass during the day and overnight they were being offered a feed of appropriate home-grown ingredients to balance the high protein and relatively low neutral detergent fibre of the grass. The by-product diet was based on ingredients available following a primary production process and not normally used for human food (March et al., 2016).

Data

Detailed animal performance data were collected on the cows routinely while they were on the genetic line x feeding systems. The present study included 546 cows with IA trait information. Of these, 256 were previously included in Banos et al., (2013). An additional 246 cohorts without IA trait information were also available and included in the bivariate analyses, resulting in a total of 792 cows with yield, reproductive and health measures. The data are summarized in Table 1 and described in further detail below.

Immune-associated Traits. Blood samples were collected on 12 separate occasions from 358 animals (2,266 total samples). Samples were collected at bi-monthly intervals between April 2013

and March 2015 and included summer and winter samplings. Blood leukocyte sub-populations in each sample were analyzed by flow cytometry to derive 9 cellular IA traits: % Peripheral blood mononuclear cells (**PBMC**), % Eosinophils, % Lymphocytes, % Monocytes, % Neutrophils, % $CD4^+$, % $CD8^+$, $CD4^+$:$CD8^+$ ratio and % $NKp46^+$.

The additional data from Banos et al., (2013) were collected at bi-monthly intervals on 5 separate occasions between July 2010 and March 2011. Cellular IA trait information from the Banos et al. (2013) data were available for animals within both genetic groups but only those on the high concentrate diet (Banos et al., 2013). This additional data accounted for approximately 25% of the total IA trait dataset.

Flow Cytometry. For flow cytometric analysis of circulating leukocyte populations, blood samples were collected into EDTA Vacutainers (BD, Franklyn, NJ). Red blood cell lysis and antibody labeling was performed in 96 well round bottomed plates as follows: 25µl of EDTA whole blood was added per well and subsequently incubated with 125µl ammonium chloride lysis buffer (0.15M NH4Cl, 10mM NaHCO3, 1mM Disodium EDTA, pH 7.4). After red blood cell lysis was complete, leukocytes were pelleted by centrifugation at 850 x g and washed twice with FACS buffer (5% FBS and 0.02% sodium azide in PBS) before incubating at 4˚C for 30 minutes in FACS buffer containing 10% heat-inactivated normal mouse serum (Invitrogen Caltag). Cell measurements were focused on cell types which had been shown to correlate with health and productivity traits in our previous study (Banos et al, 2013). Cells were then incubated at 4˚C for 30 minutes with the following monoclonal antibodies: anti-bovine CD4 conjugated to Alexa Fluor® 647 (clone CC8, mouse IgG2a, AbD Serotec), anti-bovine CD8 conjugated to R-phycoerythrin (clone CC63, mouse IgG2a, AbD Serotec) and anti-bovine CD335 conjugated to Alexa Fluor® 488 (clone AKS1, mouse IgG1, AbD Serotec). Unstained control cells and isotype stained cells (mouse IgG1 conjugated to Alexa Fluor® 488, mouse IgG2a conjugated to R-phycoerythrin, mouse IgG2a conjugated to Alexa

Fluor® 647, all eBioscience, San Diego, CA) were included on each plate. After final washes in FACS buffer then PBS, cells were fixed in 1% paraformaldehyde in PBS for 10 min at RT and then re-suspended in PBS prior to analysis on a MACSQuant® flow cytometer (Miltenyi Biotech). Data analyses were performed using FlowJo version 7.6.1 analysis software (TreeStar, San Carlos, CA). The results were expressed as a percentage of cells within the peripheral blood mononuclear cell (PBMC) population which were positive for each surface marker. In addition, differential cell counts were performed by analysis of unstained cells and identifying leukocyte populations by their size (forward scatter), granularity (side scatter), and auto-fluorescence as previously described (Lun et al., 2007).

Lactation, Feed Intake, Production and Functional Traits. A phenotypic dataset was created and matched to the immune profile of each individual animal if IA trait information was available. This data contained lactation traits recorded at the daily level and included milk yield (kg), fat and protein percentage (%), feed intake (kg), dry matter intake (kg), feed to milk ratio, dry matter to milk ratio, empty body weight (kg), live weight (kg), body condition score (0 to 5) and somatic cell count ($\times 10^3$/ml). Daily records were averaged over the week to give data for each week in milk. Information relating to record date (year, month), calving date (year, month), age at calving (months), Holstein percentage, lactation number, number of weeks in milk (**WIM**), diet group and genetic group were also included.

Health Traits. Detailed health records were available for each cow in the study population. A phenotypic dataset containing health event information (expressed as binary traits) was created and matched to the immune profile of each individual animal. Health events were grouped into 4 groups: mastitis, reproductive problems, lameness and other. Due to the low incidence of metabolic and other disorders/diseases within the Crichton herd these conditions (including ketosis, displaced abomasum,

hypocalcaemia, hypomagnesaemia, pyelonephritis etc.) were grouped into the "other" health category. Health events were then matched such that animals were scored as 0 or 1 for absence or presence of a condition or treatment within ± one week of the immune sample date. Additionally, the number of distinct mastitis, reproductive and lameness episodes per lactation was calculated for each animal. Distinct episodes were calculated according to consecutive treatments more than 7, 21 and 28 days apart for mastitis, reproductive problems and lameness respectively (Banos et al., 2013).

Fertility Traits. A fertility timeline was created for each animal and included information for each lactation such as calving date, calving interval, days to first heat, days from first to last heat, number of heats, days to first service, days from first to second service, days from first to last service, number of services, dystocia and stillbirth rate. This information was matched to each cow's immune profile in the lactation the cow was sampled for immunological analysis. Calving interval referred to the interval between the date of calving of the previous lactation and the current calving. Number of services referred to the total number of artificial inseminations before positive conception. Dystocia and stillbirth referred to the calving previous to the current lactation and were expressed as binary (0/1) traits. Dystocia was scored as of 0 for a normal calving else 1 and stillbirth was scored as 0 if calves were born alive and 1 if born dead (or died within 24 hours).

Statistical Analysis

Statistical analysis of cellular IA traits was carried out using a repeated measures mixed linear animal model with a pedigree relationship matrix fitted to account for the genetic relationships between animals:

$$y_{ijklmnopq} = \mu + F_j + G_k + W_m + T_n + Y_q + L_l + C_i + a_o + p_o + e_{ijklmnopq} \quad \textbf{(1)}$$

Where $y_{ijklmnopq}$ is the trait record; μ is the overall mean; F_j is the fixed effect of the j^{th} diet group; G_k is the fixed effect of the k^{th} genetic group; W_m is the fixed effect of the m^{th} lactation week; T_n is the fixed effect of the n^{th} assay technique, fitted to account for the variation between the methods used to generate the IA trait data; Y_q is the fixed effect of the q^{th} year by month of record interaction; L_l is the fixed effect of the l^{th} lactation number by age at calving; C_i is the fixed effect of the i^{th} year by month of calving interaction; a_o is the random additive genetic effect of the o^{th} individual cow including pedigree data (2793 animal in pedigree, see Table 1 for further details); p_o is the random permanent environmental effect of the o^{th} individual cow, fitted to account for the use of repeated measures of the same animal; and $e_{ijklmnopq}$ is the random residual effect.

Total phenotypic variances (σ_p^2), as well as corresponding additive genetic (σ_a^2), permanent environmental (σ_{pe}^2), residual (σ_e^2) variance and covariance components were estimated by the Restricted Maximum Likelihood (**REML**) approach using ASReml version 3 (Gilmour et al., 2009). Univariate models were run initially for each trait to establish the correct model (the significance levels of the fixed effects are presented in Supplementary Information Table S2) followed by a series of bivariate models to estimate the genetic/phenotypic correlations between cellular IA traits and the health, fertility and production traits. For all model outputs P-values <0.05 were considered significant. The variance components were used in the calculation of the following genetic and phenotypic parameters: the ratio of total phenotypic variance attributed to additive genetic variation (heritability, h^2); the ratio of total phenotypic variance due to the individual animal (sum of additive genetic and permanent environmental effects), *i.e.*, between-individual variation (repeatability, R); and the ratio of total phenotypic variance due to permanent environmental variance (c^2).

RESULTS

227 The data used in this study are summarized in Tables 2 (IA traits); 3 (production and
228 functional traits); and 4 (health and fertility traits). Coefficients of variation of the traits were
229 substantial and ranged from 18% (% PBMC) to 95% (% eosinophils) for IA traits; 12% (protein %)
230 to 318% (somatic cell count) for production and functional traits; and 18% (calving interval) to
231 113% (days first to last service) for fertility traits. The coefficient of variation was used as an
232 indicator of trait variability, and as seen above, marked differences in variability amongst recorded
233 traits was observed. Consistent levels of variability in these traits was observed previously (Banos et
234 al., 2013).
235
236 Total phenotypic variances (σ_p^2), as well as corresponding additive genetic (σ_a^2), permanent
237 environmental (σ_{pe}^2) and residual (σ_e^2) variance components, and their standard errors, were
238 estimated and are presented in Table 5. Estimates of heritability (h^2), between-individual variation
239 (repeatability, *R*), and the ratio of total phenotypic variance due to permanent environmental variance
240 (c^2) are also presented in Table 5. Statistically significant ($P<0.05$) heritability estimates were
241 obtained for all 9 IA traits. Heritability estimates ranged from 0.15 (% monocytes) to 0.46 (% $CD4^+$)
242 with the majority above 0.2. The highest heritabilities were observed in the T cell and NK cell
243 subsets (% $CD4^+$, % $CD8^+$, $CD4^+$:$CD8^+$ ratio and % $NKp46^+$; 0.46, 0.41, 0.43 and 0.42 respectively;
244 Table 5). Significant heritability estimates suggest these traits could be improved with selective
245 breeding.
246
247 All IA traits were shown to be repeatable and between-animal variation accounted for 18% to
248 81% of total phenotypic variance (Table 5). The most significant estimates of repeatability were
249 observed in % $CD4^+$, % $CD8^+$ $CD4^+$:$CD8^+$ ratio and % $NKp46^+$ (0.70, 0.76, 0.81 and 0.59
250 respectively, see Table 5). These estimates were higher than those previously obtained from a
251 considerably smaller dataset (Banos et al., 2013).

The permanent environmental effect was fitted to estimate any between-animal variation over and above that due to additive genetic effects. This could be due to long-term environmental effects (*e.g.*, previous diseases) and/or non-additive genetic effects (*e.g.*, epigenetic) which pertain to individual animals throughout their lives but are not passed on to the next generation. A significant ratio of total phenotypic variance due to permanent environmental variance (c^2) was found for % eosinophils, % $CD4^+$, % $CD8^+$, $CD4^+$:$CD8^+$ ratio, and % $NKp46^+$ (0.22, 0.23, 0.34, 0.38 and 0.17 respectively). For all traits, with the exception of % eosinophils, estimates for c^2 were lower than the heritability. Moreover, all traits appeared to show higher genetic variances in comparison with permanent environmental variances (% eosinophils once again being the only exception). In the case of % PBMC, % eosinophils, % lymphocytes, % monocytes and % neutrophils the largest proportion of phenotypic variance was observed in the residual variance (Table 5). Significant repeatability estimates may help to derive predictions of future animal performance. No significant estimates of c^2 were obtained for the remaining traits which may be a function of dataset size.

Correlations between Immune-Associated Traits and Production, Functional, Fertility and Health Traits

Additive genetic correlations between traits of interest to this study are presented in Tables 6 and 7 along with their corresponding standard errors. Informative phenotypic correlations are presented in Table 8. Less informative phenotypic correlations as well as permanent environmental and residual correlations are summarised in Supplementary Tables S3 to S4, S5 to S6 and S7 to S8 respectively.

Milk fat percentage was found to have a moderate positive genetic correlation with % PBMC (0.33) and % lymphocytes (0.36), and a negative association with neutrophils (-0.35). Moderate

277 negative genetic correlations were found between the $CD4^+$:$CD8^+$ ratio within the PBMC population
278 and liveweight (-0.52) and similarly, empty body weight (-0.52). Further significant genetic
279 correlations were between IA and feed conversion traits; these were low to moderate and are
280 presented in Table 6. Regarding fertility traits (Table 7) significant correlations were observed
281 between % $NKp46^+$ and stillbirth rate (0.61). Analyses yielded no significant correlations with health
282 traits (Table 7), however, the following relationships were found to be approaching significance (i.e.,
283 $0.5<P<0.1$), highlighting the requirement for further investigation: lameness episodes and % $CD8^+$ (-
284 0.51, $P=0.06$); lameness episodes and $CD4^+$:$CD8^+$ ratio (0.47, $P=0.08$); and mastitis and %
285 eosinophils (0.63, $P=0.09$).
286
287 The largest significant phenotypic correlations (Table 8) estimated between IA and
288 production traits were all negative and were between the $CD4^+$:$CD8^+$ ratio and liveweight, empty
289 body weight and body condition score, BCS, (-0.16, -0.15 and -0.11 respectively). For the fertility
290 traits a negative association between % $CD4^+$ and time between first and second service was
291 identified (-0.14) as well as a positive relationship between % monocytes and calving interval (0.10).
292 The remaining phenotypic correlations where close to zero and are summarized in Supplementary
293 Tables S3 (production and functional traits) and S4 (fertility and health).
294
295 Regarding production and functional traits, the only statistically significant permanent
296 environmental correlations were moderate, negative and between % eosinophils and milk yield (-
297 0.47), feed intake (-0.50), dry matter intake (-0.52) and metabolizable energy intake (-0.53).
298 Additionally, % $CD4^+$ was found to be negatively correlated with BCS (-0.43). In the health traits a
299 negative association between % eosinophils and reproductive episodes (-0.25) as well as a positive
300 association between % $CD4^+$ and mastitis (0.30) were noted. Finally in the case of the fertility traits
301 permanent environmental correlations were found to be moderate and negative between $CD4^+$:$CD8^+$

ratio and number of heats/services (-0.25, -0.27 respectively). Moreover, positive relationships where found to exist between % $CD8^+$ and number of services, number of heats and the time between first and last service (0.26, 0.23 and 0.23 respectively). Permanent environmental correlations may be used to develop optimal management practices regarding future animal performance (see Supplementary Tables S5 to S6 for production, fertility and health traits respectively).

Residual correlations, *i.e.*, correlations which relate to covariation unexplained by the model of analysis, were generally low or close to zero in all traits (see Supplementary Tables S7 to S8).

DISCUSSION AND CONCLUSIONS

Previously, Thompson-Crispi et al. (2012b) showed antibody- and cell-mediated immune-response traits in Holstein-Friesian dairy cows to be heritable, with estimates of 0.29 and 0.19 respectively, this was further confirmed by Heriazon et al. (2013), however, these studies focused on immune-response traits rather than the steady state measured IA traits presented here. In the present study, significant genetic and phenotypic associations were observed between T cell subsets and fertility as well as lameness events. T cell subsets such as CD4 T helper cells produce cytokines and chemokines and play an important role in immune protection, interacting with many other immune cells such as B cells, eosinophils, basophils macrophages, and neutrophils (Zhu and Paul, 2009). Earlier work by Saama et al. (2004) also highlighted the potential importance lymphocyte subsets as indicators of immune competence in dairy cattle. As highlighted above, the T cell subsets showed the most promising heritabilities, which were consistent with previous studies (Saama et al., 2004; Clapperton et al., 2009). Specifically, heritability of %$CD4^+$ has been previously reported as 0.69 in pigs (Clapperton et al., 2009). Moreover, Ahmadi et al. (2001) reported a heritability of 0.54 in humans. The Ahmadi et al. (2001) study measured actual $CD4^+$ cell counts in contrast to $CD4^+$ measured as a proportion of PBMC (*i.e.*, % $CD4^+$) as in Clapperton et al. (2009) and the present

study. Comparison of the genetic variance estimates from all three studies suggests they are similar regardless of whether total numbers or proportions are used, presumably as the numbers of PBMC/ml blood are not changing considerably between individuals.

Previously, $CD4^+$:$CD8^+$ ratio was shown to have a negative phenotypic correlation with milk somatic cell count in cows (-0.56, Banos et al., 2013). The present study estimated a genetic correlation between $CD4^+$:$CD8^+$ and SCC of -0.31 (*P=0.17*). The present study utilized a much larger dataset (four fold increase in IA records) collected over a longer period and incorporated the original data collected previously (Banos et al., 2013). Although not significant, the result suggests at a genetic level animals with lower values of $CD4^+$:$CD8^+$ ratios will have higher somatic cell counts. Moreover, a high somatic cell count in milk is often considered as an indicator of mastitis and other intra-mammary infections in cattle (Mrode and Swanson, 1996, 2003); many countries currently use SCC (or somatic cell score) to indirectly breed for mastitis resistance (Miglior et al., 2005). In the present study, the genetic correlation between SCC and mastitis was 0.67 with corresponding phenotypic correlation of 0.12. A lower value of $CD4^+$:$CD8^+$ can be indicative of a chronic infection and a higher value indicative of fighting a major/viral infection. A low $CD4^+$:$CD8^+$ ratio may potentially indicate the presence of mastitis infection, either by sequestering circulating $CD4^+$ T cells into the mammary gland (e.g., Taylor et al., 1997; Tassi et al., 2013), or preferentially expanding both circulatory and mammary populations of $CD8^+$ T cells, as these have been shown to play a key role in protection against intra-mammary infection (Denis et al., 2011). Evidence of such an association has also been reported (Park et al., 2004).

Additionally, the $CD4^+$:$CD8^+$ ratio, a cell-mediated adaptive IA trait that decreases with age (Wikby et al., 1998; Hadrup et al., 2006; Strindhall et al., 2007), has been found to exhibit a high level of heritability across species, for example 0.65 in humans (Hall et al., 2000) and; 0.64 in pigs (Flori et al., 2011a). The $CD4^+$ cells are associated with fighting against infections whereas the $CD8^+$ cells are killer cells of the immune system. The $CD4^+$:$CD8^+$ ratio gives an indication of the strength

of the immune system such that declining ratios are associated with immune dysfunction and increased risks of severe infections and malignancies (Wikby et al., 1998; Strindhall et al., 2007; Lu et al., 2015). In humans, the $CD4^+$:$CD8^+$ ratio can be used as a marker of HIV to AIDS progression (Fahey et al., 1990; Serrano-Villar et al., 2015). Other human health conditions that have been previously associated with the $CD4^+$:$CD8^+$ ratio include chronic lymphocytic leukaemia (Bartik et al., 1998; Gonzalez-Rodriguez et al., 2010), infectious mononucleosis and other viral infections (Karcheva et al., 2008; Salih, 2009), Hodgkin disease (Gupta, 1980; Poppema, 1996; Gorczyca et al., 2002; Hernandez et al., 2005), aplastic anaemia (Zhang et al., 2007), as well as neurological disorders like multiple sclerosis (Pender et al., 2014) and myasthenia gravis (Berrih et al., 1981; Matsui and Kameyama, 1986). Further, there is substantial evidence that this trait is under genetic control in mice, chickens and humans (Kraal et al., 1983; Clementi et al., 1999; Amadori et al., 1995; Myrick et al., 2002; Ewald et al., 1996).

The present study also identified a moderately strong genetic correlation between $CD4^+$:$CD8^+$ ratio and lameness (0.51, *P=0.06*) which was only identified at the phenotypic level in our previous study (Banos et al., 2013). This suggests that animals with higher steady state values of $CD4^+$:$CD8^+$ ratios are genetically predisposed to higher incidences of lameness. Additional moderate genetic correlations were found between SCC and $CD8^+$ (0.36, *P=0.12*) and monocytes (0.48, *P=0.08*) but were not statistically significant. As $CD4^+$:$CD8^+$ is useful in particular types of infections it would be interesting to explore if the relationship is consistent with different mastitis and/or lameness causing pathogens and duration of said health events.

A strong genetic correlation was found between % $NKp46^+$, a natural killer (**NK**) cell marker (Sivori et al., 1997; Storset et al., 2004) and stillbirth (0.61, *P=0.04*), which is a novel finding in cattle. This is in agreement with literature concerning human studies which have consistently identified a relationship between NK cells and reproductive outcomes, with higher percentages of NK cells within the circulating lymphocyte pool being associated with poor reproduction (Kwak-

Kim and Gilman-Sachs, 2008; Kwak-Kim et al., 2010; Seshadri and Sunkara, 2014; Michou et al., 2003). NK cells are a type of innate immune cell with potent cytotoxic activity that are important in controlling intracellular pathogens (Storset et al, 2004). Circulating NK cells can traffic into the uterus and their association with reproductive failure is thought to be due to unregulated NK-mediated cytotoxicity within the uterine environment (Kwak-Kim and Gilman-Sachs, 2008).

Previous research has demonstrated that the occurrence of metabolic and infectious disease in dairy cows classed as high immune responding (**HIR**) is lower than non-HIR cows (Thompson-Crispi et al., 2012a, 2013). Results from the present study support opinions in the literature that genetic selection of measurable immune-associated phenotypes may be possible (Thompson-Crispi et al., 2012b; Heriazon et al., 2013), could provide a useful tool in monitoring and improving disease resistance and animal health (Thompson-Crispi et al., 2014a; Mallard et al., 2015, 2011) and may not negatively impact production (Stoop et al., 2016). Results also highlight the importance of blood leukocyte subsets with respect to reproduction and fertility in dairy cows, the strong association found between stillbirth and % $NKp46^+$ is promising and gives a foundation for further investigation.

One limitation of the current study is that no functional assessment has been performed on the various leukocyte subsets measured. Many of these subsets exhibit a wide range of functional capabilities, which will be related to their previous antigenic experience (particularly for lymphocyte subsets) or other environmental and host factors (e.g. nutritional, reproductive or disease status). For example, while $CD8^+$ T cells largely target intracellular pathogens through killing of infected cells and production of antiviral cytokines (Bevan, 2004), $CD4^+$ T cells differentiate into a number of distinct helper-T cell subsets including T-helper (T_H)-1, T_H-2, T_H-9, T_H-17 and regulatory T cells, all of which exhibit different functionalities in relation to the types of pathogens they target or their role in regulating the immune response (Nakayamada et al., 2012). Thus, associations between the cellular traits used in this study and health traits may be weaker and/or absent in other study populations, and consequently the health benefits of selection for these cellular traits in dairy cattle

may be unpredictable. In future studies, IA traits involving additional cellular markers and/or immune assays which better reflect immune function (e.g. naïve vs. memory T cell markers, cytokine release profiles) should be explored. Such an approach would be similar to, but less labour intensive than that taken by other studies (Thompson-Crispi et al, 2013, 2014a), in which proposed selection is based on antibody-mediated immune response traits (broadly representing T_H-2 immunity) and cell-mediated immune response traits (broadly representing T_H-1 immunity) obtained following immunization of cattle with specific antigens.

In addition to the immunological measures of blood leukocyte subsets considered in the present study, serological immune phenotypes measurable in both bovine milk and blood may also be of value in improving the health and welfare of dairy cows. Associations between IA traits found in blood and milk have been highlighted, for example, in natural antibodies (de Klerk et al., 2015) and haptoglobin (Hiss et al., 2009). Furthermore, supporting the results of the present study serological IA traits are considered beneficial, an association between haptoglobin and mastitis has previously been highlighted (Banos et al., 2013). Moreover, obtaining data collected out with the research herd used in the present study would be advantageous and provide a means of validating our results.

In the present study we have provided evidence that cellular IA traits derived from measurable blood leukocyte populations are heritable and would permit selection for altered trait values, particularly in the case of the T cell subsets. Moreover, the associations observed between IA, health, fertility and production traits suggest that genetic selection for cellular IA traits could lead to a useful tool in improving animal health, fitness and fertility.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to BBSRC for funding this research (grant no. BB/K002260/1). Additionally, the authors also thank all staff at Crichton Farm for collecting and prompt transfer of the samples; Ian Archibald for managing the data and assisting with data extraction; Raphael Mrode for statistical input. The Langhill experiment at Crichton Dairy Research Centre, EW, TMcN and GR are supported by the Scottish Government Rural Affairs, Food and the Environment (RAFE) Strategic Research Portfolio 2016-2021.

APPENDIX

Supplementary Information accompanies this paper and is available on the Journal of Dairy Science website (www.journalofdairyscience.org)

REFERENCES

Abdel-Azim, G.A., A.E. Freeman, M.E. Kehrli, S.C. Kelm, J.L. Burton, a L. Kuck, and S. Schnell. 2005. Genetic basis and risk factors for infectious and noninfectious diseases in US Holsteins. I. Estimation of genetic parameters for single diseases and general health. *J. Dairy Sci.* 88:1199–207. doi:10.3168/jds.S0022-0302(05)72786-7.

Ahmadi, K.R., M.A. Hall, P. Norman, R.W. Vaughan, H. Snieder, T.D. Spector, and J.S. Lanchbury. 2001. Genetic determinism in the relationship between human CD4+ and CD8+ T lymphocyte populations? *Genes Immun.* 2:381–7. doi:10.1038/sj.gene.6363796.

Amadori, A., R. Zamarchi, G. De Silvestro, G. Forza, G. Cavatton, G.A. Danieli, M. Clementi, and L. Chieco-Bianchi. 1995. Genetic control of the CD4/CD8 T-cell ratio in humans. *Nat. Med.* 1:1279–1283. doi:10.1038/nm1295-1279.

Banos, G., E. Wall, M.P. Coffey, A. Bagnall, S. Gillespie, G.C. Russell, and T.N. McNeilly. 2013. Identification of Immune Traits Correlated with Dairy Cow Health, Reproduction and Productivity. *PLoS One*. 8:e65766. doi:10.1371/journal.pone.0065766.

Bartik, M.M., D. Welker, and N.E. Kay. 1998. Impairments in immune cell function in B cell chronic lymphocytic leukemia. *Semin. Oncol.* 25:27–33.

Bell, M.J., E. Wall, G. Russell, G. Simm, and A.W. Stott. 2011. The effect of improving cow productivity, fertility, and longevity on the global warming potential of dairy systems. *J. Dairy Sci.* 94:3662–3678. doi:10.3168/jds.2010-4023.

456 Berrih, S., C. Gaud, M. a Bach, H. Le Brigand, J.P. Binet, and J.F. Bach. 1981. Evaluation of T cell
457 subsets in myasthenia gravis using anti-T cell monoclonal antibodies. *Clin. Exp. Immunol.*
458 45:1–8.

459 Bevan, M.J. 2004. Helping the CD8(+) T-cell response. *Nat. Rev. Immunol.* 4:595–602.
460 doi:10.1038/nri1413.

461 Clapperton, M., S.C. Bishop, and E.J. Glass. 2005. Innate immune traits differ between Meishan and
462 Large White pigs. *Vet. Immunol. Immunopathol.* 104:131–44.
463 doi:10.1016/j.vetimm.2004.10.009.

464 Clapperton, M., A.B. Diack, O. Matika, E.J. Glass, C.D. Gladney, M.A. Mellencamp, A. Hoste, and
465 S.C. Bishop. 2009. Traits associated with innate and adaptive immunity in pigs: heritability and
466 associations with performance under different health status conditions. *Genet. Sel. Evol.* 41:54.
467 doi:10.1186/1297-9686-41-54.

468 Clapperton, M., E.J. Glass, and S.C. Bishop. 2008. Pig peripheral blood mononuclear leucocyte
469 subsets are heritable and genetically correlated with performance. *Animal*. 2:1575–84.
470 doi:10.1017/S1751731108002929.

471 Clementi, M., P. Forabosco, a Amadori, R. Zamarchi, G. De Silvestro, E. Di Gianantonio, L. Chieco-
472 Bianchi, and R. Tenconi. 1999. CD4 and CD8 T lymphocyte inheritance. Evidence for major
473 autosomal recessive genes. *Hum. Genet.* 105:337–342.

474 Collard, B.L., P.J. Boettcher, J.C. Dekkers, D. Petitclerc, and L.R. Schaeffer. 2000. Relationships
475 between energy balance and health traits of dairy cattle in early lactation. *J. Dairy Sci.* 83:2683–
476 2690. doi:10.3168/jds.S0022-0302(00)75162-9.

477 Denis, M., S.J. Lacy-Hulbert, B.M. Buddle, J.H. Williamson, and D.N. Wedlock. 2011.
478 Streptococcus uberis-specific T cells are present in mammary gland secretions of cows and can
479 be activated to kill S. uberis. *Vet. Res. Commun.* 35:145–156. doi:10.1007/s11259-011-9462-1.

480 Ewald, S.J., Y.Y. Lien, L. Li, and L.W. Johnson. 1996. B-haplotype control of CD4/CD8 subsets and
481 TCR V beta usage in chicken T lymphocytes. *Vet. Immunol. Immunopathol.* 53:285–301.
482 doi:10.1016/S0165-2427(96)05613-9.

483 Fahey, J.L., J.M. Taylor, R. Detels, B. Hofmann, R. Melmed, P. Nishanian, and J. V Giorgi. 1990.
484 The prognostic value of cellular and serologic markers in infection with human
485 immunodeficiency virus type 1. *N. Engl. J. Med.* 322:166–72.
486 doi:10.1056/NEJM199001183220305.

487 Flori, L., Y. Gao, D. Laloë, G. Lemonnier, J.-J. Leplat, A. Teillaud, A.-M. Cossalter, J. Laffitte, P.
488 Pinton, C. de Vaureix, M. Bouffaud, M.-J. Mercat, F. Lefèvre, I.P. Oswald, J.-P. Bidanel, and
489 C. Rogel-Gaillard. 2011a. Immunity traits in pigs: substantial genetic variation and limited
490 covariation. *PLoS One*. 6:e22717. doi:10.1371/journal.pone.0022717.

491 Flori, L., Y. Gao, I.P. Oswald, F. Lefevre, M. Bouffaud, M.-J. Mercat, J.-P. Bidanel, and C. Rogel-
492 Gaillard. 2011b. Deciphering the genetic control of innate and adaptive immune responses in
493 pig: a combined genetic and genomic study. *BMC Proc.* 5:S32. doi:10.1186/1753-6561-5-S4-
494 S32.

Gilmour, A., B. Gogel, B. Cullis, and R. Thompson. 2009. ASReml user guide release 3.0. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Glass, E.J., P.M. Preston, A. Springbett, S. Craigmile, E. Kirvar, G. Wilkie, and C.G.D. Brown. 2005. Bos taurus and Bos indicus (Sahiwal) calves respond differently to infection with Theileria annulata and produce markedly different levels of acute phase proteins. *Int. J. Parasitol.* 35:337–47. doi:10.1016/j.ijpara.2004.12.006.

Gonzalez-Rodriguez, A.P., J. Contesti, L. Huergo-Zapico, A. Lopez-Soto, A. Fernández-Guizán, A. Acebes-Huerta, A.J. Gonzalez-Huerta, E. Gonzalez, C. Fernandez-Alvarez, and S. Gonzalez. 2010. Prognostic significance of CD8 and CD4 T cells in chronic lymphocytic leukemia. *Leuk. Lymphoma*. 51:1829–1836. doi:10.3109/10428194.2010.503820.

Gorczyca, W., J. Weisberger, Z. Liu, P. Tsang, M. Hossein, C.D. Wu, H. Dong, J.Y.L. Wong, S. Tugulea, S. Dee, M.R. Melamed, and Z. Darzynkiewicz. 2002. An Approach to Diagnosis of T-cell Lymphoproliferative Disorders by Flow Cytometry. *Clin. Cytom.* 50:177–190. doi:10.1002/cyto.10003.

Gupta, S. 1980. Subpopulations of human T lymphocytes. XVI. Maldistribution of T cell subsets associated with abnormal locomotion of T cells in untreated adult patients with Hodgkin's disease. *Clin. Exp. Immunol.* 42:186–195.

Hadrup, S.R., J. Strindhall, T. Køllgaard, T. Seremet, B. Johansson, G. Pawelec, P. thor Straten, and A. Wikby. 2006. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. *J. Immunol.* 176:2645–2653. doi:176/4/2645 [pii].

Hall, M.A., K.R. Ahmadi, P. Norman, H. Snieder, a J. MacGregor, R.W. Vaughan, T.D. Spector, and J.S. Lanchbury. 2000. Genetic influence on peripheral blood T lymphocyte levels. *Genes Immun.* 1:423–7. doi:10.1038/sj.gene.6363702.

Heriazon, A., M. Quinton, F. Miglior, K.E. Leslie, W. Sears, and B.A. Mallard. 2013. Phenotypic and genetic parameters of antibody and delayed-type hypersensitivity responses of lactating Holstein cows. *Vet. Immunol. Immunopathol.* 154:83–92. doi:10.1016/j.vetimm.2013.03.014.

Hernandez, O., T. Oweity, and S. Ibrahim. 2005. Is an increase in CD4/CD8 T-cell ratio in lymph node fine needle aspiration helpful for diagnosing Hodgkin lymphoma? A study of 85 lymph node FNAs with increased CD4/CD8 ratio. *Cytojournal*. 2:14. doi:10.1186/1742-6413-2-14.

Hiss, S., C. Weinkauf, S. Hachenberg, and H. Sauerwein. 2009. Short communication: Relationship between metabolic status and the milk concentrations of haptoglobin and lactoferrin in dairy cows during early lactation. *J. Dairy Sci.* 92:4439–4443. doi:10.3168/jds.2008-1632.

Huijps, K., T.J. Lam, and H. Hogeveen. 2008. Costs of mastitis: facts and perception. *J. Dairy Res.* 75:113–20. doi:10.1017/S0022029907002932.

Karcheva, M., T. Lukanov, S. Gecheva, V. Slavcheva, G. Veleva, and R. Nachev. 2008. Infectious mononucleosis-Diagnostic potentials. *In* Journal of IMAB-Annual Proceeding (scientific papers) Book 1. Peytchinski Gospodin Iliev, Pleven. 9–13.

De Klerk, B., B.J. Ducro, H.C.M. Heuven, I. den Uyl, J. a. M. van Arendonk, H.K. Parmentier, and J.J. van der Poel. 2015. Phenotypic and genetic relationships of bovine natural antibodies binding keyhole limpet hemocyanin in plasma and milk. *J. Dairy Sci.* 98:2746–2752. doi:10.3168/jds.2014-8818.

Van Knegsel, A.T.M., M. Hostens, G. de Vries Reilingh, A. Lammers, B. Kemp, G. Opsomer, and H.K. Parmentier. 2012. Natural antibodies related to metabolic and mammary health in dairy cows. *Prev. Vet. Med.* 103:287–97. doi:10.1016/j.prevetmed.2011.09.006.

Koeck, a, F. Miglior, J. Jamrozik, D.F. Kelton, and F.S. Schenkel. 2013. Genetic associations of ketosis and displaced abomasum with milk production traits in early first lactation of Canadian Holsteins. *J. Dairy Sci.* 96:4688–96. doi:10.3168/jds.2012-6408.

Kraal, G., I.L. Weissman, and E.C. Butcher. 1983. Genetic control of T-cell subset representation in inbred mice. *Immunogenetics*. 18:585–92.

Kwak-Kim, J., and A. Gilman-Sachs. 2008. Clinical implication of natural killer cells and reproduction. *Am. J. Reprod. Immunol.* 59:388–400. doi:10.1111/j.1600-0897.2008.00596.x.

Kwak-Kim, J., S. Smith, M. Chapman, and G. Sacks. 2010. Detailed analysis of peripheral blood natural killer (NK) cells in women with recurrent miscarriage. *Hum. Reprod.* 25:52–58. doi:10.1093/humrep/dep349.

Lu, W., V. Mehraj, K. Vyboh, W. Cao, T. Li, and J.P. Routy. 2015. CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. *J. Int. AIDS Soc.* 18. doi:10.7448/IAS.18.1.20052.

Lun, S., G. Aström, U. Magnusson, and K. Ostensson. 2007. Total and differential leucocyte counts and lymphocyte subpopulations in lymph, afferent and efferent to the supramammary lymph node, during endotoxin-induced bovine mastitis. *Reprod. Domest. Anim.* 42:126–134. doi:10.1111/j.1439-0531.2006.00741.x.

Mallard, B.A., H. Atalla, S. Cartwright, B.C. Hine, B. Hussey, M. Paibomesai, K.A. Thompson-Crispi, and L. Wagter-Lesperance. 2011. Genetic and epigenetic regulation of the bovine immune system: Practical implications of the high immune response technology. *In* Proc. National Mastitis Council 50th Annual Meeting. National Mastitis Council, Madison, WI. 53–61.

Mallard, B.A., M. Emam, M. Paibomesai, K.A. Thompson-Crispi, and L. Wagter-Lesperance. 2015. Genetic selection of cattle for improved immunity and health. *Jpn. J. Vet. Res.* 63:S37–S44. doi:10.14943/jjvr.63.suppl.s37.

March, M.D., L. Toma, a. W. Stott, and D.J. Roberts. 2016. Modelling phosphorus efficiency within diverse dairy farming systems - Pollutant and non-renewable resource? *Ecol. Indic.* 69:667–676. doi:10.1016/j.ecolind.2016.05.022.

Matsui, M., and M. Kameyama. 1986. A double-label flow cytometric analysis of the simultaneous expression of OKT4 and Leu2a antigens on circulating T lymphocytes in myasthenia gravis. *J. Neuroimmunol.* 11:311–319. doi:10.1016/0165-5728(86)90084-6.

McDougall, S., D.G. Arthur, M. a Bryan, J.J. Vermunt, and a M. Weir. 2007. Clinical and bacteriological response to treatment of clinical mastitis with one of three intramammary antibiotics. *N. Z. Vet. J.* 55:161–70. doi:10.1080/00480169.2007.36762.

Michou, V.I., P. Kanavaros, V. Athanassiou, G.B. Chronis, S. Stabamas, and V. Tsilivakos. 2003. Fraction of the peripheral blood concentration of CD56+/CD16−/CD3− cells in total natural killer cells as an indication of fertility and infertility. *Fertil. Steril.* 80:691–697. doi:10.1016/S0015-0282(03)00778-7.

Miglior, F., B.L. Muir, and B.J. Van Doormaal. 2005. Selection indices in Holstein cattle of various countries. *J. Dairy Sci.* 88:1255–1263. doi:10.3168/jds.S0022-0302(05)72792-2.

Mrode, R.A., and G.J.T. Swanson. 1996. Genetic and statistical properties of somatic cell count and its suitability as an indirect means of reducing the incidence of mastitis in dairy cattle. *Anim. Breed. Abstr.* 64:847–856.

Mrode, R.A., and G.J.T. Swanson. 2003. Estimation of genetic parameters for somatic cell count in the first three lactations using random regression. *Livest. Prod. Sci.* 79:239–247. doi:10.1016/S0301-6226(02)00169-0.

Myrick, C., R. DiGuisto, J. DeWolfe, E. Bowen, J. Kappler, P. Marrack, and E.K. Wakeland. 2002. Linkage analysis of variations in CD4:CD8 T cell subsets between C57BL/6 and DBA/2. *Genes Immun.* 3:144–150. doi:10.1038/sj.gene.6363819.

Nakayamada, S., H. Takahashi, Y. Kanno, and J.J. O'Shea. 2012. Helper T cell diversity and plasticity. *Curr. Opin. Immunol.* 24:297–302. doi:10.1016/j.coi.2012.01.014.

Oltenacu, P.A., and D.M. Broom. 2010. The Impact of Genetic Selection for Incresed Milk Yield on the Welfare of Dairy Cows. *Univ. Fed. Anim. Welf.* 19:39–49.

Park, Y.H., Y.S. Joo, J.Y. Park, J.S. Moon, S.H. Kim, N.H. Kwon, J.S. Ahn, W.C. Davis, and C.J. Davies. 2004. Characterization of lymphocyte subpopulations and major histocompatibility complex haplotypes of mastitis-resistant and susceptible cows. *J. Vet. Sci.* 5:29–39.

Pender, M.P., P. a Csurhes, C.M. Pfluger, and S.R. Burrows. 2014. Deficiency of CD8+ effector memory T cells is an early and persistent feature of multiple sclerosis. *Mult. Scler.* 20:1825–32. doi:10.1177/1352458514536252.

Poppema, S. 1996. Immunology of Hodgkin's disease. *Baillieres. Clin. Haematol.* 9:447–457. doi:10.1016/S0950-3536(96)80020-5.

Pritchard, T., M.P. Coffey, R. Mrode, and E. Wall. 2013. Genetic parameters for production, health, fertility and longevity traits in dairy cows. *Animal.* 7:34–46. doi:10.1017/S1751731112001401.

Pryce, J.E., B.L. Nielsen, R.F. Veerkamp, and G. Simm. 1999. Genotype and feeding system effects and interactions for health and fertility traits in dairy cattle. *Livest. Prod. Sci.* 57:193–201. doi:10.1016/S0301-6226(98)00180-8.

Pryce, J.E., M.D. Royal, P.C. Garnsworthy, and I.L. Mao. 2004. Fertility in the high-producing dairy cow. *Livest. Prod. Sci.* 86:125–135. doi:10.1016/S0301-6226(03)00145-3.

608 Saama, P.M., J.B. Jacob, M.E. Kehrli, a E. Freeman, S.C. Kelm, a L. Kuck, R.J. Tempelman, and
609 J.L. Burton. 2004. Genetic variation in bovine mononuclear leukocyte responses to
610 dexamethasone. *J. Dairy Sci.* 87:3928–37. doi:10.3168/jds.S0022-0302(04)73532-8.

611 Salih, S.M. 2009. Lymphocyte subsets phenotype in patients with infectious mononucleosis. *J. Fac.*
612 *Med. Baghdad*. 51:227–231.

613 Serrano-Villar, S., T. Sainz, and S. Moreno. 2015. Monitoring the CD4/CD8 ratio: a promising
614 indicator of disease progression in HIV-infected individuals? *Future Virol.* 10:1–4.
615 doi:10.2217/fvl.14.85.

616 Seshadri, S., and S.K. Sunkara. 2014. Natural killer cells in female infertility and recurrent
617 miscarriage: A systematic review and meta-analysis. *Hum. Reprod. Update*. 20:429–438.
618 doi:10.1093/humupd/dmt056.

619 Sivori, S., M. Vitale, L. Morelli, L. Sanseverino, R. Augugliaro, C. Bottino, L. Moretta, and A.
620 Moretta. 1997. P46, a Novel Natural Killer Cell-Specific Surface Molecule That Mediates Cell
621 Activation. *J. Exp. Med.* 186:1129–1136. doi:10.1084/jem.186.7.1129.

622 Stoop, C., K.A. Thompson-Crispi, S. Cartwright, and B.A. Mallard. 2016. Short communication:
623 Variation in production parameters among Canadian Holstein cows classified as high, average,
624 and low immune responders. *J. Dairy Sci*. 99:1–5. doi:10.3168/jds.2015-10145.

625 Storset, A.K., S. Kulberg, I. Berg, P. Boysen, J.C. Hope, and E. Dissen. 2004. NKp46 defines a
626 subset of bovine leukocytes with natural killer cell characteristics. *Eur. J. Immunol.* 34:669–
627 676. doi:10.1002/eji.200324504.

628 Strindhall, J., B.O. Nilsson, S. Löfgren, J. Ernerudh, G. Pawelec, B. Johansson, and A. Wikby. 2007.
629 No Immune Risk Profile among individuals who reach 100 years of age: Findings from the
630 Swedish NONA immune longitudinal study. *Exp. Gerontol.* 42:753–761.
631 doi:10.1016/j.exger.2007.05.001.

632 Tassi, R., T.N. McNeilly, J.L. Fitzpatrick, M.C. Fontaine, D. Reddick, C. Ramage, M. Lutton, Y.H.
633 Schukken, and R.N. Zadoks. 2013. Strain-specific pathogenicity of putative host-adapted and
634 nonadapted strains of Streptococcus uberis in dairy cattle. *J. Dairy Sci.* 96:5129–5145.
635 doi:10.3168/jds.2013-6741.

636 Taylor, B.C., R.G. Keefe, J.D. Dellinger, Y. Nakamura, J.S. Cullor, and J.L. Stott. 1997. T cell
637 populations and cytokine expression in milk derived from normal and bacteria-infected bovine
638 mammary glands. *Cell. Immunol.* 182:68–76. doi:10.1006/cimm.1997.1215.

639 Thompson-Crispi, K.A., H. Atalla, F. Miglior, and B.A. Mallard. 2014a. Bovine mastitis: Frontiers
640 in immunogenetics. *Front. Immunol.* 5:1–10. doi:10.3389/fimmu.2014.00493.

641 Thompson-Crispi, K.A., B. Hine, M. Quinton, F. Miglior, and B.A. Mallard. 2012a. Short
642 communication: Association of disease incidence and adaptive immune response in Holstein
643 dairy cows. *J. Dairy Sci.* 95:3888–93. doi:10.3168/jds.2011-5201.

644 Thompson-Crispi, K.A., F. Miglior, and B.A. Mallard. 2013. Incidence rates of clinical mastitis
645 among Canadian Holsteins classified as high, average, or low immune responders. *Clin.*
646 *Vaccine Immunol.* 20:106–12. doi:10.1128/CVI.00494-12.

647 Thompson-Crispi, K.A., M. Sargolzaei, R. Ventura, M. Abo-Ismail, F. Miglior, F. Schenkel, and
648 B.A. Mallard. 2014b. A genome-wide association study of immune response traits in Canadian
649 Holstein cattle. *BMC Genomics*. 15:559. doi:10.1186/1471-2164-15-559.

650 Thompson-Crispi, K.A., A. Sewalem, F. Miglior, and B.A. Mallard. 2012b. Genetic parameters of
651 adaptive immune response traits in Canadian Holsteins. *J. Dairy Sci.* 95:401–9.
652 doi:10.3168/jds.2011-4452.

653 Veerkamp, R.F., G. Simm, and J.D. Oldham. 1994. Effects of interaction between genotype and
654 feeding system on milk production, feed intake, efficiency and body tissue mobilization in dairy
655 cows. *Livest. Prod. Sci.* 39:229–241. doi:10.1016/0301-6226(94)90202-X.

656 Wikby, A., P. Maxson, J. Olsson, B. Johansson, and F.G. Ferguson. 1998. Changes in CD8 and CD4
657 lymphocyte subsets, T cell proliferation responses and non-survival in the very old: The
658 Swedish longitudinal OCTO-immune study. *Mech. Ageing Dev.* 102:187–198.
659 doi:10.1016/S0047-6374(97)00151-6.

660 Zhang, Q., Q. Li, J.-W. Xu, A.-M. Zhang, X.-C. Xu, and Z.-M. Zhai. 2007. Clinical significance of
661 detection of T-cell subgroups in patients with aplastic anemia. *Zhongguo shi yan xue ye xue za*
662 *zhi*. 15:1046–1049.

663 Zhu, J., and W.E. Paul. 2009. CD4 T cells : fates , functions , and faults. *Immunobiology*. 112:1557–
664 1569. doi:10.1182/blood-2008-05-078154.

665

666

667 **Table 1.** Description of phenotype dataset used in all model analyses

Description	Total
Weekly production and functional phenotypic records	92,153
Weekly cellular immune-associated, health and fertility records	3,581
Animals in data set	792
Animals with immune data	546
Animals with phenotypic data only	246
Lactations	3[1]
Years (2005-2015)	10
Animals in pedigree	2,793
Sires	539
Dams	1,813
Generations	7

668 [1] 1,785 total lactations. Note: lactations ≥ 3 are grouped into the lactation 3 class.

669

670

671 **Table 2**. Descriptive statistics of the 9 cellular immune-associated traits obtained via flow cytometric analysis

Trait	No. Records	Min	Max	Mean	Std. Dev	CV[1] (%)
% PBMC[2, 3]	2,266	18.00	89.50	58.39	10.24	17.54
% Eosinophils[3]	2,266	0.07	35.20	3.61	3.43	95.06
% Lymphocytes[3]	2,265	7.70	79.70	44.25	12.35	27.90
% Monocytes[3]	2,265	3.03	55.40	13.99	8.25	58.98
% Neutrophils[3]	2,266	8.09	81.10	37.76	10.10	26.74
% $CD4^+$ [4]	2,232	3.39	46.00	25.52	6.28	24.61
% $CD8^+$ [4]	2,260	2.51	28.00	11.29	3.42	30.28
$CD4^+$:$CD8^+$ ratio	2,232	0.48	6.12	2.38	0.73	30.67
% $NKp46^+$ [4]	2,262	0.01	16.50	2.32	1.58	67.95

672 [1] Coefficient of variation

673 [2] % Peripheral Blood Mononuclear Cells

674 [3] % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

675 [4] % of PBMC that were CD4, CD8 and NKp46 positive

676

677

678 **Table 3.** Descriptive statistics of the 12 production and functional traits

Trait	No. Records	Min	Max	Mean	Std. Dev	CV[1] (%)
Milk (kg)	90,750	3.00	65.54	28.71	9.10	31.71
Fat (%)	72,433	0.23	9.94	3.78	0.71	18.83
Protein (%)	72,433	0.20	6.65	3.21	0.39	12.00
Feed intake (kg)	64,970	2.21	182.31	42.39	11.71	27.63
Dry matter intake (kg)	64,970	1.03	57.27	16.62	5.22	31.39
Metabolizable energy intake (MJ)	64,970	10.47	641.47	186.56	59.15	31.70
Empty body weight (kg)	88,345	237.00	782.00	483.00	67.50	13.98
Live weight (kg)	88,345	310.00	953.00	605.00	79.51	13.14
Body condition score (0-5)	69,703	0.50	4.25	2.11	0.43	20.33
Somatic cell count ($x10^3$/ml)	74,288	2.67	7,865.00	110.64	351.76	317.93
Feed intake:Milk (ratio)	64,919	0.07	271.50	1.61	1.98	122.92
Dry matter intake:Milk (ratio)	64,919	0.03	136.56	0.62	0.84	135.82

679 [1] Coefficient of variation

680

681

682

683 **Table 4.** Descriptive statistics of the 3 health traits and 9 fertility traits

Health Trait			Incidence[1]	Mean[2]	Std. Dev [2]	Max[2]
Mastitis			0.01	0.17	0.38	10
Reproductive problems			0.12	0.83	0.59	11
Lameness			0.12	0.61	0.59	12
Fertility Trait	**No. Records**	**Min**	**Max**	**Mean**	**Std. Dev**	**CV[3] (%)**
Calving interval (days)	663	189	737	404.41	74.15	18.34
Days to first heat (days)	855	2	205	58.64	30.68	52.32
Days first last heat (days)	855	0	731	86.33	93.48	108.28
Number of heats	861	0	15	3.76	2.79	74.04
Days to first service (days)	850	4	205	66.66	25.95	38.92
Days first second service (days)	634	1	206	33.71	24.13	71.58
Days first last service (days)	850	0	662	75.72	85.68	113.15
Number of services	861	0	14	3.41	2.65	77.70
Dystocia score (0/1)	861	-	-	0.22	0.41	-
Stillbirth score (0/1)	861	-	-	0.09	0.29	-

684 [1] Proportion of cows experiencing a health event on the week of immune sampling. Measured as a binary trait

685 [2] Based on number of distinct episodes per lactation

686 [3] Coefficient of variation

687

688

Table 5. Results from univariate analysis. Additive genetic (σ_a^2), permanent environmental (σ_{pe}^2), residual (σ_e^2), and phenotypic variances (σ_p^2), with standard errors, are presented for the 9 cellular immune-associated traits. Heritability estimates (h^2), ratio of permanent environmental variance (c^2) and repeatability (R), with standard errors are also provided. Statistically significant values (P<0.05) are given in bold.

Trait	σ_a^2	σ_{pe}^2	σ_e^2	σ_p^2	h^2	c^2	R
% PBMC[1, 2]	**22.45**	3.21	**50.42**	**76.08**	**0.30**	0.04	**0.34**
	(5.828)	(3.997)	(1.725)	(3.510)	(0.065)	(0.053)	(0.030)
% Eosinophils[2]	**1.18**	**1.56**	**4.41**	**7.16**	**0.17**	**0.22**	**0.38**
	(0.490)	(0.423)	(0.151)	(0.320)	(0.065)	(0.059)	(0.028)
% Lymphocytes[2]	**23.66**	0.22	**44.60**	**68.48**	**0.35**	0.00	**0.35**
	(5.655)	(3.679)	(1.563)	(3.331)	(0.071)	(0.054)	(0.032)
% Monocytes[2]	**1.19**	0.29	**6.52**	**8.00**	**0.15**	0.04	**0.18**
	(0.416)	(0.327)	(0.227)	(0.298)	(0.049)	(0.041)	(0.026)
% Neutrophils[2]	**20.62**	3.42	**51.80**	**75.85**	**0.27**	0.05	**0.32**
	(5.403)	(3.766)	(1.772)	(3.369)	(0.064)	(0.050)	(0.030)
% $CD4^+$[3]	**8.49**	**4.26**	**5.56**	**18.31**	**0.46**	**0.23**	**0.70**
	(2.258)	(1.531)	(0.193)	(1.261)	(0.101)	(0.090)	(0.023)
% $CD8^+$[3]	**4.14**	**3.44**	**2.45**	**10.04**	**0.41**	**0.34**	**0.76**
	(1.126)	(0.828)	(0.085)	(0.677)	(0.095)	(0.088)	(0.018)
$CD4^+$:$CD8^+$[3]	**0.21**	**0.19**	**0.10**	**0.50**	**0.43**	**0.38**	**0.81**
	(0.066)	(0.048)	(0.003)	(0.036)	(0.112)	(0.105)	(0.015)
% $NKp46^+$[3]	**0.55**	**0.22**	**0.55**	**1.33**	**0.42**	**0.17**	**0.59**
	(0.135)	(0.092)	(0.019)	(0.080)	(0.085)	(0.073)	(0.027)

[1] % Peripheral Blood Mononuclear Cells

[2] % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

[3] % of PBMC that were CD4, CD8 and NKp46 positive

Table 6. Additive genetic correlations of immune-associated traits with production traits. Significant correlations (P<0.05) are given in bold.

Trait	% PBMC[1,2]	% Eosinophils[2]	% Lymphocytes[2]	% Monocytes[2]	% Neutrophils[2]	% CD4+[3]	% CD8+[3]	CD4+:CD8+[3]	% NKp46+[3]
Milk (kg)	-0.14 (0.196)	0.25 (0.251)	-0.23 (0.186)	0.02 (0.235)	0.07 (0.202)	-0.01 (0.205)	-0.05 (0.210)	0.18 (0.220)	-0.01 (0.195)
Fat (%)	**0.33 (0.147)**	0.15 (0.205)	**0.36 (0.137)**	0.11 (0.187)	**-0.35 (0.149)**	0.12 (0.159)	0.09 (0.156)	-0.13 (0.162)	0.09 (0.152)
Protein (%)	0.19 (0.151)	0.03 (0.204)	0.19 (0.143)	0.13 (0.187)	-0.18 (0.155)	0.04 (0.162)	-0.06 (0.166)	0.05 (0.175)	0.22 (0.153)
Feed intake (kg)	-0.16 (0.195)	0.19 (0.255)	-0.16 (0.189)	-0.03 (0.234)	0.09 (0.201)	-0.16 (0.193)	-0.06 (0.207)	-0.09 (0.215)	0.30 (0.184)
Dry matter intake (kg)	-0.18 (0.210)	0.25 (0.269)	-0.19 (0.203)	0.01 (0.255)	0.09 (0.217)	-0.23 (0.207)	-0.15 (0.223)	-0.02 (0.236)	0.29 (0.201)
Metabolizable energy intake (MJ)	-0.17 (0.214)	0.24 (0.276)	-0.19 (0.207)	0.01 (0.259)	0.09 (0.221)	-0.26 (0.208)	-0.15 (0.227)	-0.04 (0.239)	0.29 (0.205)
Empty body weight (kg)	-0.32 (0.172)	0.22 (0.215)	-0.26 (0.168)	-0.11 (0.205)	0.25 (0.175)	-0.02 (0.173)	0.33 (0.176)	**-0.52 (0.172)**	0.18 (0.160)
Live weight (kg)	-0.31 (0.172)	0.23 (0.215)	-0.25 (0.168)	-0.11 (0.205)	0.24 (0.175)	-0.02 (0.173)	0.33 (0.176)	**-0.52 (0.172)**	0.18 (0.161)
Body condition score (0-5)	-0.17 (0.205)	-0.04 (0.243)	-0.08 (0.194)	-0.21 (0.234)	0.18 (0.208)	0.22 (0.199)	0.22 (0.201)	-0.20 (0.209)	-0.03 (0.186)
Somatic cell count ($x10^3$/ml)	0.17 (0.233)	-0.13 (0.294)	0.09 (0.220)	0.48 (0.262)	-0.14 (0.230)	0.13 (0.226)	0.36 (0.232)	-0.31 (0.238)	-0.03 (0.213)
Feed intake:Milk (ratio)	**0.25 (0.028)**	-0.03 (0.149)	N.E.	-0.13 (0.166)	-0.19 (0.115)	**0.07 (0.009)**	0.14 (0.095)	N.E.	**0.24 (0.103)**
Dry matter intake:Milk (ratio)	N.E.	-0.02 (0.157)	**0.33 (0.120)**	-0.02 (0.144)	**-0.24 (0.028)**	**0.08 (0.011)**	0.06 (0.135)	N.E.	**0.31 (0.020)**

[1] % Peripheral Blood Mononuclear Cells

[2] % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

[3] % of PBMC that were CD4, CD8 and NKp46 positive

Table 7. Additive genetic correlations of immune-associated traits with fertility and health traits. Significant correlations (P<0.05) are given in bold.

Trait	% PBMC[1, 2]	% Eosinophils[2]	% Lymphocytes[2]	% Monocytes[2]	% Neutrophils[2]	% CD4+ [3]	% CD8+ [3]	CD4+:CD8+ [3]	% NKp46+ [3]
Calving interval	0.07	0.37	-0.12	0.39	-0.14	-0.11	-0.42	0.40	0.18
(days)	(0.290)	(0.388)	(0.281)	(0.332)	(0.297)	(0.300)	(0.329)	(0.349)	(0.290)
Days to first heat	0.24	0.32	0.09	0.61	-0.35	-0.36	-0.19	-0.07	-0.20
(days)	(0.330)	(0.394)	(0.304)	(0.412)	(0.355)	(0.311)	(0.324)	(0.333)	(0.291)
Days first last	0.20	0.63	0.08	0.43	-0.36	-0.50	-0.84	0.87	0.07
heat (days)	(0.586)	(0.779)	(0.531)	(0.836)	(0.702)	(0.718)	(1.532)	(1.526)	(0.549)
Number of heats	0.20	0.40	0.10	0.42	-0.28	-0.10	-0.23	0.42	0.09
	(0.306)	(0.388)	(0.295)	(0.376)	(0.315)	(0.322)	(0.359)	(0.386)	(0.304)
Days to first	0.22	0.28	0.06	0.56	-0.30	0.03	0.17	-0.08	-0.23
service (days)	(0.285)	(0.344)	(0.268)	(0.358)	(0.297)	(0.286)	(0.286)	(0.303)	(0.264)
Days first last	0.15	0.72	0.01	0.39	-0.37	-0.63	N.E.	N.E.	0.57
service (days)	(0.741)	(1.067)	(0.640)	(1.107)	(1.062)	(0.949)			(2.150)
Number of	0.17	0.38	0.09	0.36	-0.25	-0.16	-0.28	0.40	0.17
services	(0.295)	(0.375)	(0.284)	(0.352)	(0.303)	(0.309)	(0.349)	(0.366)	(0.301)
Dystocia score	-0.13	-0.06	-0.03	-0.14	0.11	-0.29	-0.22	-0.02	0.23
(0/1)	(0.237)	(0.308)	(0.231)	(0.275)	(0.241)	(0.234)	(0.254)	(0.264)	(0.243)
Stillbirth score	-0.10	-0.67	-0.01	-0.42	0.27	0.22	0.44	-0.32	**0.61**
(0/1)	(0.330)	(0.368)	(0.310)	(0.397)	(0.352)	(0.330)	(0.344)	(0.369)	**(0.278)**
Mastitis	-0.07	0.63	-0.03	0.10	-0.27	-0.32	0.14	0.09	0.03
	(0.377)	(0.360)	(0.379)	(0.498)	(0.514)	(0.355)	(0.399)	(0.457)	(0.362)
Lameness	0.02	-0.39	-0.01	0.01	0.10	-0.05	0.08	-0.08	0.06
	(0.267)	(0.283)	(0.259)	(0.308)	(0.261)	(0.277)	(0.267)	(0.287)	(0.252)
Other condition	-0.53	N.E.	-0.38	-0.65	0.24	0.01	0.53	-0.47	-0.22
	(0.606)		(0.658)	(0.728)	(0.598)	(0.666)	(0.642)	(0.874)	(0.660)
Mastitis episodes	0.05	0.23	-0.06	0.66	-0.12	-0.10	0.21	-0.08	0.05
	(0.406)	(0.478)	(0.369)	(0.571)	(0.431)	(0.395)	(0.401)	(0.419)	(0.373)
Lameness	-0.02	0.27	-0.06	0.05	-0.01	-0.15	-0.51	0.47	0.28
episodes	(0.236)	(0.337)	(0.228)	(0.281)	(0.236)	(0.243)	(0.261)	(0.266)	(0.235)

[1] % Peripheral Blood Mononuclear Cells

[2] % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

[3] % of PBMC that were CD4, CD8 and NKp46 positive

Table 8. Phenotypic correlations of immune-associated traits with production, health and fertility traits. Significant correlations (P<0.05) are given in bold

Trait	% Eosinophils[2]	% Monocytes[2]	% Neutrophils[2]	% $CD4^+$ [3]	$CD4^+$:$CD8^+$	% $NKp46^+$ [3]
Milk (kg)	-0.04 (0.033)	**0.08 (0.029)**	-0.01 (0.032)	0.04 (0.039)	0.03 (0.040)	0.02 (0.037)
Empty body weight (kg)	**0.08 (0.039)**	**-0.08 (0.034)**	0.01 (0.038)	-0.08 (0.049)	**-0.15 (0.049)**	-0.02 (0.046)
Live weight (kg)	**0.08 (0.039)**	**-0.08 (0.034)**	0.01 (0.038)	-0.08 (0.049)	**-0.16 (0.049)**	-0.02 (0.046)
Body condition score (0-5)	0.06 (0.033)	**-0.06 (0.029)**	-0.03 (0.032)	-0.07 (0.040)	**-0.11 (0.041)**	-0.10 (0.037)
Somatic cell count ($x10^3$/ml)	0.04 (0.031)	-0.05 (0.028)	0.05 (0.031)	-0.00 (0.037)	-0.05 (0.038)	**-0.01 (0.035)**
Feed intake:Milk (ratio)	0.01 (0.018)	-0.01 (0.023)	-0.01 (0.018)	-0.01 (0.012)	**-0.02 (0.000)**	0.01 (0.018)
Dry matter intake:Milk (ratio)	0.01 (0.020)	-0.00 (0.020)	**-0.01 (0.000)**	**-0.01 (0.000)**	N.E.	0.02 (0.014)
Calving interval (days)	-0.05 (0.044)	**0.10 (0.036)**	0.01 (0.042)	-0.01 (0.053)	0.03 (0.055)	-0.00 (0.049)
Days first second service (days)	**-0.04 (0.006)**	N.E.	0.04 (0.035)	**-0.14 (0.046)**	-0.02 (0.050)	0.01 (0.044)
Lameness	0.03 (0.021)	0.01 (0.022)	-0.01 (0.021)	**0.05 (0.021)**	0.02 (0.020)	-0.02 (0.021)
Other condition	**-0.04 (0.019)**	-0.01 (0.020)	0.02 (0.020)	0.03 (0.019)	0.03 (0.018)	**0.04 (0.019)**

[1] % Peripheral Blood Mononuclear Cells

[2] % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils

[3] % of PBMC that were CD4, CD8 and NKp46 positive